European Journal of Medicinal Chemistry 224 (2021) 113682

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Synthesis and computational studies of novel fused pyrimidinones as a promising scaffold with analgesic, anti-inflammatory and COX inhibitory potential

Mona F. Said [a, **], Hanan H. Georgey [a, b, *], Eman R. Mohammed [a]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt, Kasr El-Aini Street, Cairo, P.O. Box 11562, Egypt
[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Heliopolis University for Sustainable Development, Cairo, 11777, Egypt

ARTICLE INFO

Article history:
Received 19 April 2021
Received in revised form
29 June 2021
Accepted 30 June 2021
Available online 3 July 2021

Keywords:
Fused pyrimidinones
Analgesic
Anti-inflammatory
QSAR study
Pharmacophore

ABSTRACT

Addressing the global need for the development of safe and potent NSAIDs, new series of oxadiazolo and thiadiazolo fused pyrmidinones were synthesized and initially tested for their analgesic activity. All tested compounds showed promising analgesic activity compared with the reference standard indomethacin. Moreover, anti-inflammatory activity evaluation, ulcerogenic liability, and *in vitro* COX-1, COX-2 enzyme inhibition assays were also performed for the most active derivatives. The methoxyphenyl piperazinyl derivative **3d** showed analgesic activity surpassing indomethacin with protection of 100%, and 83%; respectively. Also **3d** showed good anti-inflammatory activity with relatively lower ulcer index compared with other tested compounds, and potent COX-1 and COX-2 inhibitory activity with IC_{50} = 0.140, 0.007 μm, respectively, and with a selectivity index of 20.00 which was better than the reference standards and the other tested congeners. Additionally, compounds **3b**, **3g** and **3h** revealed moderate selectivity (SI = 3.53, 3.70 and 5.87, respectively). Moreover, *in silico* physicochemical parameters revealed that the new fused pyrimidinones demonstrated promising pharmacokinetic properties. Furthermore, computational studies in form of 2D-quantitative structure-activity relationship (2D-QSAR) and 3D-pharmacophore confirmed the potential analgesic properties of the new target compounds.

1. Introduction

The demand for new peripheral analgesics and anti-inflammatory agents in the market is still considered a challenge; as they are not only used in managing inflammation and pain, but help also in the symptomatic treatment of various disorders, for instance: cancer, gout, cardiovascular diseases, etc. Henceforth, investigating compounds which are able to treat both acute and chronic pain is a pivotal aspect of pharmaceutical research [1–3].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are still some of the most widely used classes of therapeutic drugs, primarily because they could be accessed as either over the counter (OTC) or prescription sales e.g. indomethacin (see Fig. 1) [4,5]. Additionally, they are used to treat an array of inflammatory disorders of mild and moderate ranges of pain [6,7]. Non-selective NSAIDs are clinically used under certain restrictions, particularly with patients who have a history of peptic ulcer, as they are accompanied with primary and secondary insult effects. They act by depriving both isoforms COX-1 and COX-2 of the cyclooxygenase enzyme which leads to hindering the production of cytoprotective prostaglandins (PGs). Accordingly, selective COX-2 inhibitors have been developed in order to overcome this obstacle through the inhibition of PGs synthesis at the site of inflammation [8,9]. However, there is an increased incidence of cardiovascular side effects due to the decrease in the protective prostacyclin (PGI_2) production [10,11]. Fortunately, extensive studies have proven that the cardiac side effects are not a necessary physiological side effect which results from all the drugs falling under that umbrella; but instead related to intrinsic chemical structure of a minor group of them [12]. Consequently, there is a crucial need to discover a new generation of effective and safe analgesic COX inhibitors with moderate potency.

* Corresponding author. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt, Kasr El-Aini Street, Cairo, P.O. Box 11562, Egypt.
** Corresponding author.
E-mail addresses: mona.abdelnoor@pharma.cu.edu.eg (M.F. Said), hanan.hana@pharma.cu.edu.eg, hanan.hana@pharma.cu.edu.eg (H.H. Georgey).

https://doi.org/10.1016/j.ejmech.2021.113682
0223-5234/

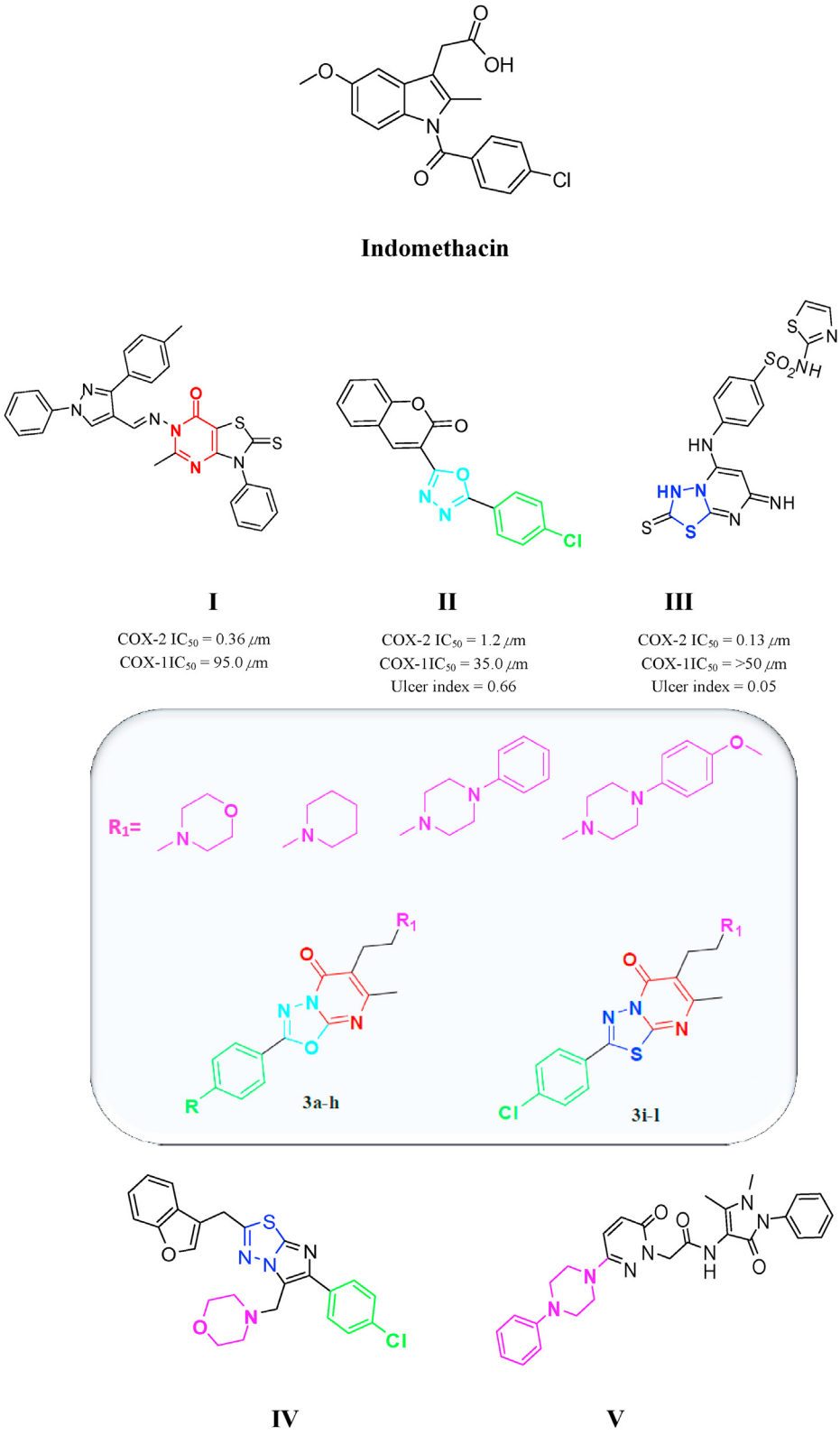


Fig. 1. Reported compounds **I**–**V** and design strategy of the new fused pyrimidinone derivatives as peripheral analgesics and anti-inflammatory agents.

In fact, there is a high similarity between COX-1 and COX-2 active site topology. Drug candidates should bear different hydrophobic moieties for proper nonspecific interactions with the enzyme hydrophobic regions responsible for the binding [2]. Minor changes within the structure, lipophilicity, and hydrogen-bonding properties, can have tremendous effects on binding, activity and selectivity [13,14].

In recent years, pyrimidine and fused pyrimidinones have shown diverse biological activities and have been reported as potent analgesics and anti-inflammatory agents with low

ulcerogenicity [15–18]; compound **I** was reported as a safe, potent and selective anti-inflammatory, analgesic agent (COX-2 IC_{50} = 0.36 μm, COX-1 IC_{50} = 95.0 μm) [17]. Additionally, several studies have confirmed that the replacement of the carboxylic acid moiety in NSAIDs with less acidic oxadiazole or thiadiazole led to increase the activity and decrease the ulcerogenicity [19–22], In addition, researchers have reported 1,3,4-oxadiazoles [19], and 1,3,4,-thiadiazoles [22] as potent analgesic and anti-inflammatory agents with lower ulcer effect such as compounds **II** and **III** which exerted COX-2 IC_{50} = 1.2 μm and 0.13 μm with ulcer index = 0.66 and 0.05, respectively. Also many compounds bearing morpholine or substituted piperazine moieties (for example **IV** [23], and **V** [1,24]) were reported to have potent analgesic and anti-inflammatory activity (see Fig. 1).

Motivated by all the aforementioned facts, and in our attempt to develop new, safer and potent peripheral analgesic and anti-inflammatory agents, some novel fused [1,3,4]oxadiazolo [3,2-a] pyrimidin-5-one **3a-h**, and [1,3,4]thiadiazolo [3,2-a]pyrimidin-5-one **3i-l** were synthesized. These sets of compounds were hybridized with different secondary amines (see Fig. 1).

The newly prepared compounds have been evaluated for their peripheral analgesic activity. Compounds showing potent analgesic activity were chosen to be tested for their anti-inflammatory activity, ulcerogenicity, acute toxicity, and ability to inhibit COX-1 and COX-2 isozymes by *in vitro* COX inhibition assays.

Two dimensional-quantitative structure activity relationship (2D-QSAR) studies were also performed to correlate between the structures of the new target compounds and their pharmacological activity. Additionally, 3D-pharmacophore studies were done to identify the bio-active centers of the compounds determining the biological properties. Furthermore, key physicochemical parameters were calculated to indicate their compliance with Lipinski's rule of five [25] to investigate their pharmacokinetic properties and drug-likeness.

2. Results and discussion

2.1. Chemistry

The synthetic pathway for the preparation of the target compounds is illustrated in Scheme 1. The key starting materials **1a-c** were prepared according to the reported methods [26–28]. Pyrimidinones **2a-c** were furnished by the reaction of starting materials **1a-c** with 2-acetylbutyrolactone in presence of $POCl_3$. ^{1}HNMR spectra of **2a-c** revealed the appearance of a singlet signal at 2.51–2.95 ppm corresponding to $\underline{CH}_3$ protons, and two triplets of 2 $\underline{CH}_2$ protons at 3.08–3.10 ppm and 3.88–3.92 ppm. Reaction of key intermediates **2a-c** with different secondary amines, namely; morpholine, piperidine, phenyl piperazine, and *p*-methoxyphenyl piperazine; afforded compounds **3a-d**. All new derivatives were confirmed by spectral and elemental analysis as mentioned in details in the experimental part.

Reagents and reaction condition. (***i***) $POCl_3$, reflux, 2h. (***ii***) DMF, K_2CO_3, reflux 12 h.

2.2. Biological evaluation

2.2.1. In vivo screening

2.2.1.1. Analgesic activity. The new target compounds **3a-l** were evaluated for their analgesic activity using *p*-benzoquinone-induced writhing method in mice reported by Okun et al. [29] and indomethacin was used as a reference standard. The number of protected animals by the tested compounds and the reference drug are recorded in Table 1. Results revealed that all the tested compounds showed potent to moderate analgesic activity compared to the reference drug indomethacin; the *p*-methoxyphenyl piperazine analog **3d** revealed potency higher than that of indomethacin.

2.2.1.2. Structural activity relationship. In order to study the SAR of the newly designed compounds; we compared the analgesic activity concerning three major parameters; firstly, comparing the analgesic activity of oxadiazolo to thiadiazolo derivatives, it was revealed that, the presence of oxygen is more preferable for activity than its bioisostere, less electronegative sulfur atom; oxadiazolo derivatives (**3e-3h**) revealed protection of 4–5 animals out of 6, while thiadiazolo derivatives (**3i-3l**) protection ranged from 3 to 4 animals out of 6 only. Additionally, only oxadiazolo derivative **3d** exhibited protection of 6 animals out of 6 (100% protection). Secondly, regarding the side chain; generally it was obvious that the phenyl piperazino derivatives (**3c**, **3g**, and **3k**) and *p*-methoxyphenyl piperazino derivatives (**3d**, **3h**, and **3l**) were more active than the relatively more hydrophilic piperidino derivatives (**3b**, **3f**, and **3j**) and morpholino derivatives (**3a**, **3e**, and **3i**); it is worthily mentioned that the *p*-methoxyphenyl piperazine analog **3d** revealed 100% protection. Finally, regarding the presence or absence of *p*-chloro substituent in oxadiazolo derivatives; it was observed that the presence of *p*-chloro led to increased activity only in morpholino derivative **3e** (66 % protection) and phenyl pepirazino **3g** (83% protection) compared to the unsubstituted ones **3a** (50% protection), and **3c** (50% protection) (see Fig. 2).

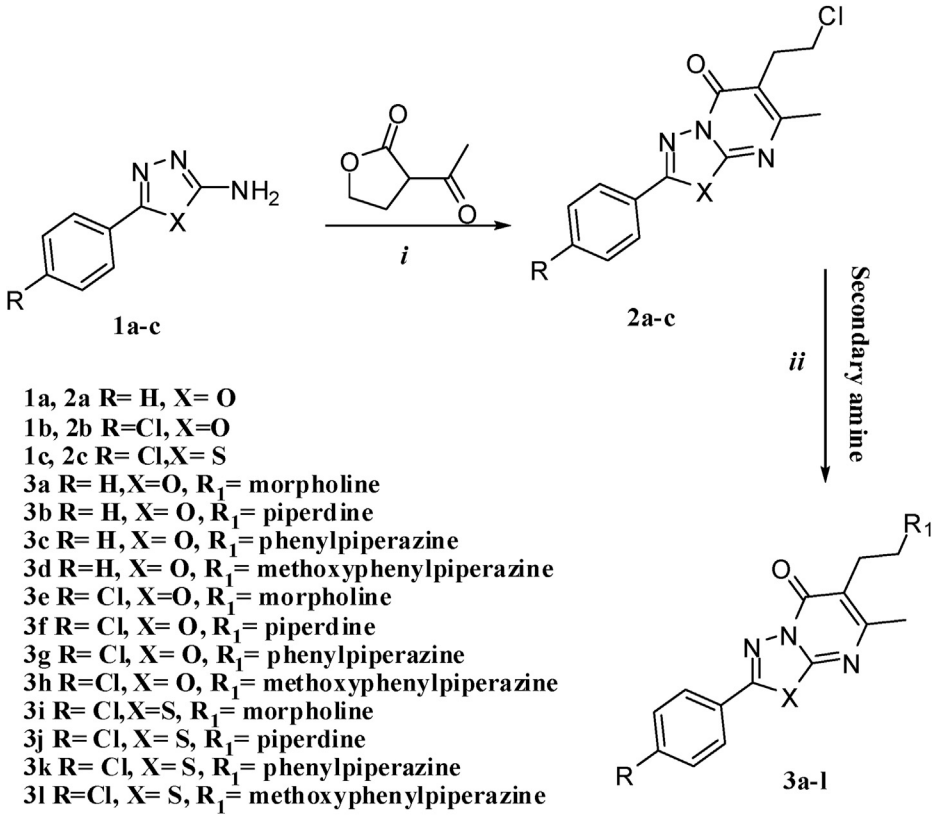
2.2.1.3. Toxicological study. Toxicological study of the most active compounds **3b**, **3d**, **3g**, and **3h** was determined using standardized method [30]. All tested compounds showed a high safety margin (up to 5 folds of the used analgesic dose) and intraperitoneal injection of doses less than 140 μm/kg body weight of the tested compounds failed to kill any mice after their observation 24h.

2.2.1.4. Anti-inflammatory activity. Compounds **3b**, **3d**, **3g**, and **3h** that showed the best analgesic activity were selected for evaluation of anti-inflammatory activity using carrageenan-induced rat paw edema method reported by Winter et al. [31] using indomethacin as reference standard. Results recorded in Table 2 revealed that all compounds that showed promising analgesic activity, also exhibited promising anti-inflammatory activity. Piperidino derivative **3b** and phenyl piperazino derivative **3g** were found to be the most potent ones with potency 104% and 129%, respectively at 2h effect.

2.2.1.5. Ulcerogenic liability. The ulcerogenic effect of compounds **3b**, **3d**, **3g**, and **3h** that exhibited a promising analgesic and anti-inflammatory activities and reference drug indomethacin was evaluated using the reported method of Meshali et al. [32], and the ulcer index was calculated according to the reported method [33] (Table 3) (Supplementary Fig. S1). From the recorded results it was observed that, all the tested compounds for ulcerogenic liability revealed better GIT tolerance than indomethacin. It is worthily mentioned that methoxyphenyl piperazine **3d** was found to display the lowest ulcerogenic effect as indicated from its ulcer index 3.80.

2.2.2. In vitro COX inhibition assay

The most active compounds **3b**, **3d**, **3g**, and **3h**, in addition to the reference drugs celecoxib and indomethacin were subjected to *in vitro* COX-1 and COX-2 inhibition assays, using COX-1 Inhibitor Screening Kit (Fluorometric) Cat. #K548 and COX-2 Inhibitor Screening Kit (Fluorometric) Cat.#K547 according to the manufacturer's instructions. IC_{50} for COX-1 and COX-2 and IC_{50} ratio of the tested compounds, celecoxib and indomethacin are recorded in Table 4. Results revealed that the tested compounds showed a promising potency as COX inhibitors. They showed moderate COX-1 inhibition (0.140 μm - 0.350 μm) compared to that of the



Scheme 1. Synthesis of target compounds **1**–**3**.

Table 1
Analgesic activity of indomethacin, and the new compounds in mice (n = 6) at dose (28 μm/Kg).

Cpd. No.	No. of protected animals/6	%Protection	% Potency**
Control	0	0	0
Indomethacin	5/6	83	100
3a	3/6	50	60
3b	5/6	83	100
3c	3/6	50	60
3d	6/6	100	120
3e	4/6	66	80
3f	4/6	66	80
3g	5/6	83	100
3h	5/6	83	100
3i	4/6	66	80
3j	3/6	50	60
3k	4/6	66	60
3l	4/6	66	60

**%Potency = %Protection relative to indomethacin.

reference drugs celecoxib and indomethacin (0.091 μm and 0.012 μm) respectively; and a promising COX-2 inhibition (0.007–0.099 μm) better than indomethacin (0.082 μm) but lower than celecoxib (0.006 μm). Three compounds (**3b**, **3g** and **3h**) revealed moderate selectivity (SI = 3.53, 3.70 and 5.87, respectively). COX-2 IC_{50} of methoxyphenyl piperazine **3d** was found to be 11-fold more potent than indomethacin (IC_{50} = 0.082 μm) and nearly equipotent with celecoxib (IC_{50} = 0.006). Moreover, it showed a high selectivity index (SI = 20.00) more than that expressed by celecoxib (SI = 15.17). Thus, it is worth to mention that compound **3d** was found to be the most potent, selective and safe COX-2 inhibitor.

2.3. Physicochemical parameters

Calculation of the different molecular properties showed that all the target compounds obeyed the "rule of five" [25] criteria. It was also found that all the target compounds revealed high % ABS, ranging from 82% to 91% topological polar surface area (TPSA) range of 50–76 A^2, and number of rotatable bonds (nrotb) range of 4–6 which are all in the acceptable range for drug-like molecules (Table S1). The most active compounds **3b**, **3d**, **3g**, and **3h** showed good % ABS = 87, 82, 85 and 82%, respectively. Moreover, they revealed miLogP≤5 (2.99, 3.73, 4.35 and 4.41), with a molecular weight less than 500, obeying Lipinski rule of five. This confirmed that the new compounds processed promising analgesic activity and remarkable physicochemical profile as well.

2.4. In silico studies

2.4.1. 2D-QSAR study

Analgesic properties of the synthesized agents (**3a-3l**) were undertaken by QSAR (CODESSA-Pro software) [34] study for validating the observed data and identifying the most important descriptor (physicochemical parameters) governing the biological properties [35,36]. Good internally validated three descriptor QSAR model was obtained (R^2 = 0.874, R^2cvOO = 0.761, R^2cvMO = 0.804) (Table 5). The BMLR-QSAR model attained is due to the non-diverse short data set observed due to the current study. The model covered a good range of bio-properties (observed protection = 50 – 100, predicted protection = 49.37–98.73). In other words, the model is accessible/useable for mild to excellent biologically active agents but only limited to compounds of the chemical scaffold(s) utilized ([1,3,4]thiadiazolo[3,2-*a*]pyrimidines and their thio-analogues, non-diverse data set). Attempts were made for

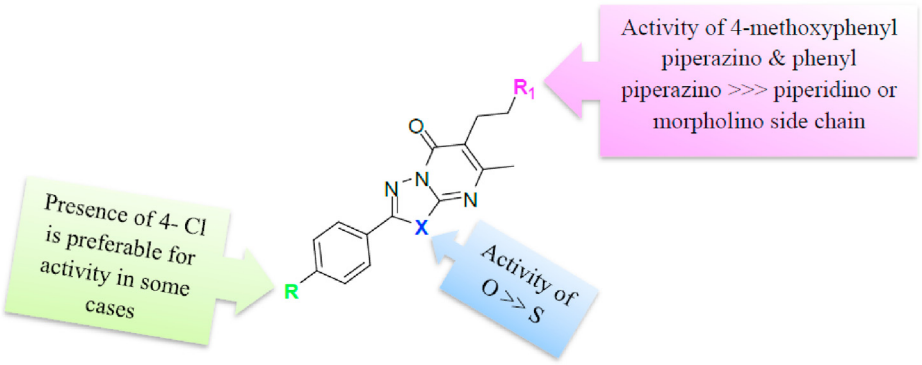


Fig. 2. SAR study of the newly synthesized derivatives.

Table 2
Anti-inflammatory effect of indomethacin, and four chosen synthesized compounds on carrageenan induced edema of the hind paw in rats (n = 5) at dose (28 μm/Kg).

Cpd. No.	Edema (mm) ± SEM (%inhibition of inflammation)		%Potency**
	2h	3h	
Control	1.60 ± 0.12	1.64 ± 0.04	-
Indomethacin	0.42 ± 0.13* (**73.29**)	0.55 ± 0.09* (**66.46**)	100
3b	0.38 ± 0.15* (**76.25**)	0.70 ± 0.03* (**56.16**)	104
3d	0.43±.0.16* (**73.12**)	0.62 ± 0.16* (**61.70**)	99
3g	0.08 ± 0.05* (**94.75**)	0.48 ± 0.18* (**70.73**)	129
3h	0.43 ± 0.16* (**73.12**)	0.14 ± 0.14* (**91.00**)	99

Statistical analysis was carried out by one-way ANOVA test.
* Significance difference from the control value at p < 0.001.
** % Potency = % edema thickness inhibition relative to that of the standard reference (indomethacin) at 2h effect.

Table 3
Ulcerogenic effect of indomethacin and four chosen synthesized compounds in rats (n = 5) at dose (28 μm/Kg).

Cpd. No.	% incidence divided by 10	Average no. of ulcer	Average severity	Ulcer index
Control	0	0	0	0
Indomethacin	8	2.60	1.18	11.78
3b	4	2.20	1.20	7.40
3d	2	0.80	1.00	3.80
3g	4	0.60	1.00	5.60
3h	4	0.80	1.00	5.80

Table 4
IC_{50} for COX-1 and COX-2 and selectivity index (SI) ratio of the tested compounds, celecoxib and indomethacin.

Cpd. No.	IC_{50} (μm)[a]		SI[b]
	COX-1	COX-2	
Celecoxib	0.091 ± 1.06	0.006 ± 0.08	15.17
Indomethacin	0.012 ± 0.12	0.082 ± 0.82	0.14
3b	0.350 ± 8.44	0.099 ± 0.98	3.53
3d	0.140 ± 2.38	0.007 ± 0.11	20.00
3g	0.148 ± 2.70	0.040 ± 0.48	3.70
3h	0.350 ± 1.41	0.015 ± 0.19	5.87

[a] IC_{50} is the concentration needed to cause 50% inhibition of COX-1 and COX-2 enzymatic activity.
[b] SI (Selectivity Index) = COX-1 IC_{50}/COX-2 IC_{50}.

extension of the data points through searching the literature for previously reported analogues (utilizing the same experimental technique and the same chemical scaffold) were unsuccessful.

Maximum e-e repulsion for atom O (semi-empirical descriptor *t*

Table 5
Descriptors of the QSAR model for the analgesic active agents.

ID	Coefficient	*S*	*T*	Descriptor
0	−2.88326	0.417	−6.921	Intercept
D_1	0.0134384	0.002	7.040	Max. e-e repulsion for atom O
D_2	−0.00299184	0.001	−3.919	Tot. dipole of the molecule
D_3	−0.000308645	5.624E-005	−5.488	Vib. enthalpy (300K)/natoms

$N = 12$, $n = 3$, $R^2 = 0.874$, R^2cvOO = 0.761, R^2cvMO = 0.804, $F = 18.496$
$s^2 = 1.999e\text{-}006$
1/(% Protection) = -2.88326 + (0.0134384 x D_1) − (0.00299184 x D_2) − (0.000308645 x D_3)

"criterion value" = 7.040) positively correlated to the attained QSAR model describing the 1/property "% protection". In other words the high descriptor value affords low biological property (% protection) as exhibited by compounds **3a** and **3b** (descriptor values = 222.431, 221.959 corresponding to predicted % protection = 53.39, 77.49, respectively). This descriptor supports what mentioned in the SAR section concerning the high potency of oxadiazolyl containing-heterocycles (**3e-3h**) relative to the thiadiazolyl analogues (**3i-3l**) (Table 6).

Total dipole of the molecule is also a semi-empirical descriptor negatively correlated to the QSAR model determining 1/% protection of the molecule. So, the high descriptor value predicting high potent analog. This is exhibited in compounds **3a** and **3b** (descriptor values = 2.979, 3.367 corresponding to predicted % protection = 53.39, 77.49, respectively).

Vibrational enthalpy (300K)/natoms is a thermodynamic descriptor also negatively correlated to the QSAR model (coefficient = −0.0003). The higher descriptor value the higher estimated efficacy of the molecule (due to the negative sign of the descriptor) as viewed by compounds **3a** and **3d** (descriptor values = 253.408, 268.904 corresponding to predicted % protection = 53.39, 98.73, respectively).

Results in (Table 7, Fig. 3) show that the predicted biological properties are comparable with their experimentally observed qualitative values. This adds a robust support for the statistical internal validations attained ($F = 18.496$ $s^2 = 1.999e\text{-}006$). Due to limited data points of this study, internal validation seems the most suitable technique for the current study.

2.4.2. 3D pharmacophore study

3D-pharmacophore is a computational technique accessible for medicinal chemical studies to identify the bio-active centers of the compounds determining the biological properties [37,38]. The synthesized agents with potential analgesic properties were undertaken by Discovery Studio 2.5 software for identifying the 3D-pharmacophic hypothesis describing the bio-observations

Table 6
Molecular descriptor values of the QSAR model for the tested compounds.

Compd.	Descriptors[c]		
	D_1	D_2	D_3
3a	222.431	2.979	253.408
3b	221.959	3.367	247.975
3c	222.464	2.609	255.772
3d	222.220	3.31	268.904
3e	222.480	1.813	272.694
3f	222.042	1.541	264.708
3g	222.037	1.344	267.226
3h	222.127	1.119	280.963
3i	222.034	1.446	269.935
3j	222.676	2.005	268.565
3k	222.586	2.443	277.264
3l	222.569	1.879	283.868

[c] D_1 = Max. e-e repulsion for atom O, D_2 = Tot. dipole of the molecule, D_3 = Vib. enthalpy (300K)/natoms.

Table 7
Observed and estimated analgesic properties for the tested compounds according to the BMLR-QSAR model.

Compd.	1/(observed protection)	Observed protection	1/(estimated protection)	Estimated protection	Error[d]
3a	0.020	50	0.019	53.39	−3.39
3b	0.012	83	0.013	77.49	5.51
3c	0.020	50	0.020	51.15	−1.15
3d	0.010	100	0.010	98.73	1.27
3e	0.015	66	0.017	59.06	6.94
3f	0.015	66	0.014	69.81	−3.81
3g	0.012	83	0.014	71.10	11.90
3h	0.012	83	0.012	85.42	−2.42
3i	0.015	66	0.013	77.59	−11.59
3j	0.020	50	0.020	49.37	0.63
3k	0.015	66	0.015	66.45	−0.45
3l	0.015	66	0.014	69.09	−3.09

[d] Error is the difference between the observed and estimated property.

(standard technique, structure optimization by CHARMm, partial charge by Momany-Rone). 3D-pharmacophore with 4 chemical features (two hydrogen bonding acceptors "HBA-1, HBA-2", one positive ionisable "PosIon" and one hydrophobic "H") was obtained due to the tested compounds ([1,3,4]thiadiazolo[3,2-*a*]pyrimidines

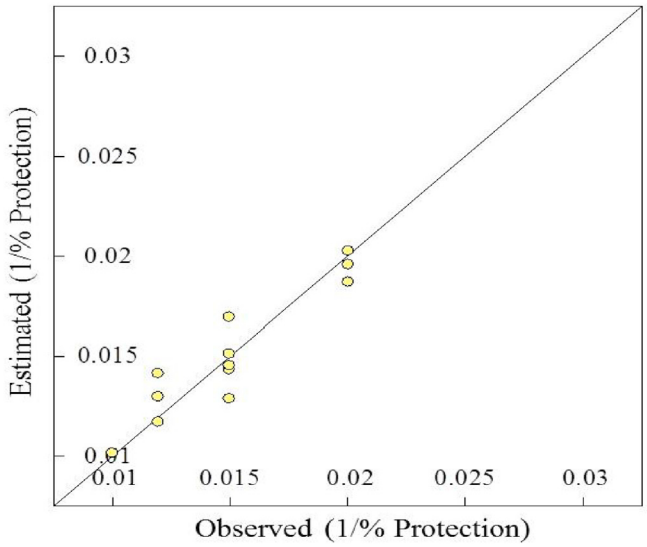


Fig. 3. QSAR plot representing the observed versus predicted 1/% protection of the tested compounds.

and their thio-analogues, non-diverse data set) with potential analgesic properties (Supplementary Fig. S2). All the tested compounds show a uniform mapping in the generated 3D-pharmacophore with variable fitness affording variable estimated bioproperties (Table 8, Supplementary Fig. S3). The oxygen/sulfur atom and the carbonyl (*C*-5) of the heterocyclic system are aligned with HBA-1 and HBA-2, respectively. The (nu)substituted phenyl ring attached at *C*-2 of the heterocycle is aligned with the hydrophobic function, while the nitrogen atom of the cyclic-amino residue is aligned with the positive ionisable. Results in Table 8 reveal the comparable estimated properties due to the 3D-pharmacophoic hypothesis with their observed qualitative values.

Fitness of the sulfur/oxygen atom of the thiadiazolyl/oxadiazolyl heterocycle with the HBA-1 supports its role in controlling the bioproperties. This observation coincides with that mentioned in the SAR due to the higher analgesic properties of oxadiazolyl containing-compounds (**3e**-**3h**) that showed estimated % protection ranges from 78.73 to 66.28 relative to that of thiadiazoyl analogues (**3i**-**3l**) that showed estimated % protection ranges from 70.67 to 48.39. Additionally, The higher potencies of 4-(4-methoxyphenyl)piperazinyl containing-compounds (**3d**, **3h**) which exhibited estimated % protection = 82.83 and 73.69, respectively relative to the 4-phenylpiperazinyl containing-analogues (**3c**, **3g**) which showed estimated % protection = 68.72 and 68.99, respectively are also supported by the hypothesized 3D-pharmacophoric model. This is also mentioned the SAR. The most potent analog **3d** (observed % protection = 100) revealed the highest estimated % protection 82.83 (Fig. S3). In other words the pharmacophoric model preserves the potencies of the tested compounds among each other.

3. Conclusion

This current study revealed that, the newly synthesized oxadiazolo and thiadiazolo fused pymidinones could be considered as promising building blocks for future research to develop potent analgesic and anti-inflammatory agents with moderate selectivity and minimal side effects. Pharmacological screening showed that all of tested compounds revealed a good analgesic activity in some cases surpassing that of indomethacin. The most potent ones (**3b**, **3d**, **3g**, and **3h**) exhibited good anti-inflammatory activity ranged from 94.75 to 73.12 %inhibition, with lower ulcerogenic liabilities compared with indomethacin. Also, *in vitro* COX-1, COX-2 enzyme inhibition assay showed a reasonable potency towards COX-1 and COX-2 with a good selectivity index. Three compounds (**3b**, **3g** and **3h**) revealed moderate selectivity (SI = 3.53, 3.70 and 5.87, respectively). The methoxyphenyl piperazinyl derivative **3d** exhibited the most potent analgesic activity with 100% protection surpassing that of indomethacin (83.33 %protection) and showed good anti-inflammatory activity with relatively lower ulcer index

Table 8
Observed and estimated biological properties of the tested compounds according to the 3D-pharmacophoic models.

Compd.	Observed % protection	Estimated % protection	Fit value
3a	50	61.08	7.923
3b	83	77.52	7.820
3c	50	68.72	7.872
3d	100	82.83	7.791
3e	66	66.28	7.888
3f	66	78.73	7.813
3g	83	68.99	7.870
3h	83	73.69	7.842
3j	50	48.39	8.024
3k	66	70.67	7.860
3l	66	62.61	7.913

compared to indomethacin and other tested compounds. It showed potent COX-1 and COX-2 inhibitory activity with IC_{50} = 0.188, 0.008 μm, respectively and selectivity index of 20.00 which was better than the reference standards and the other tested congeners. These novel fused pyrimidinones compounds also possessed promising pharmacokinetic properties indicated through calculated key physicochemical parameters and absorption percentages. Moreover, 2D-QSAR studies revealed a good internally validated and statistically significant model can investigate some expectedly potent compounds. Also, all the tested compounds show a uniform mapping in the generated 3D-pharmacophore with 4 chemical features with variable fitness (utilized non diverse heterocyclic synthesized compounds) affording variable estimated bio-properties.

4. Experimental

All chemicals were purchased from VWR International Merck, or Sigma-Aldrich, Germany. Melting points were uncorrected and were carried out by open capillary tube method using Stuart SMP3 Melting Point Apparatus. Elemental Microanalyses were carried out at the Regional Centre for Mycology and Biotechnology, Al-Azhar University. Infrared spectra were recorded on Shimadzu Infrared Spectrometer IR Affinity-1 (FTIR- 8400S-Kyoto-Japan), and expressed in wave number (cm^{-1}). ^1H NMR and ^{13}C NMR Spectra were recorded on Bruker High Performance Digital FT-NMR Spectrometer Avance III 400 MHz, ^{13}C, 100 MHz NMR spectrometer. The spectra were run at 400 MHz in deuterated chloroform ($CDCl_3$) or dimethylsulfoxide (DMSO-d_6). Chemical shifts were expressed in δ units and were related to that of the solvents. As for the proton magnetic resonance, D_2O was carried out for NH and OH exchangeable protons. Mass spectra were recorded using Shimadzu Gas Chromatograph Mass spectrometer-Qp 2010 plus (Japan) or ISQLT single quadrapole Mass spectrometer at The Regional Centre for Mycology and Biotechnology, Al-Azhar University. All the reactions were monitored by TLC using silica gel F254 plates (Merck), using chloroform: methanol 9.5:0.5 or pure chloroform as eluting system and were visualized by UV-lamp. Compounds **1a-c** [26–28], were prepared according to reported methods.

4.1. Chemistry

4.1.1. General procedure for the synthesis of compound (2a-c)

A mixture of compounds **1a**, **1b** or **1c** (0.05mol), 2-acetylbutyrolactone (0.05mol) and $POCl_3$ (15 mL) was heated under reflux for 2h. then excess $POCl_3$ was distilled under reduced pressure and the residue was triturated with ice water. The obtained suspension was neutralized with 2 M ammonia; crude product was filtered, dried, and crystallized from ethanol.

*4.1.1.1. 6-(2-Chloroethyl)-7-methyl-2-phenyl-5H- [1,3,4]oxadiazolo [3,2-a]pyrimidin-5-one **(2a)**.* Yellow crystals, yield 38%; m.p. 188–190 °C; IR (KBr, cm^{-1}): 3066 (CH aromatic), 2924, 2854 (CH aliphatic), 1716 (C═O), 1566 (C═C); ^1H NMR ($CDCl_3$, 400 MHz): δ 2.59 (s, 3H, CH_3), 3.08 (t, 2H, CH_2–CH_2–Cl, J = 6.1 Hz), 3.88 (t, 2H, CH_2–CH_2–Cl, J = 6.1 Hz), 7.49–7.52 (m, 3H, aromatic H), 8.25–8.28 (m, 2H, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.3, 28.9, 42.8, 109.6, 127.4, 128.7, 129.0, 131.0, 154.3, 156.8, 162.0, 162.6; EIMS, *m/z*: 289 (M^+); Anal. Calcd. For $C_{14}H_{12}ClN_3O_2$ (289.72): C, 58.04; H, 4.17; N, 14.50. Found: C, 58.61; H, 3.98; N, 14.68.

*4.1.1.2. 6-(2-Chloroethyl)-2-(4-chlorophenyl)-7-methyl-5H- [1,3,4] oxadiazolo [3,2-a]pyrimidin-5-one **(2b)**.* Buff crystals, yield 73%; m.p. 218–220 °C; IR (KBr, cm^{-1}): 3032 (CH aromatic), 2924, 2854 (CH aliphatic), 1766 (C═O), 1496 (C═C); ^1H NMR (DMSO, 400 MHz): δ 2.51 (s, 3H, CH_3), 3.87 (t, 2H, CH_2–CH_2–Cl, J = 7.2 Hz), 3.92 (t, 2H, CH_2–CH_2–Cl, J = 7.1 Hz), 7.56 (d, 2H, aromatic H, J = 8.6 Hz), 7.79 (d, 2H, aromatic, J = 8.6 Hz); ^{13}C NMR (DMSO, 100 MHz): δ 16.4, 16.5, 62.4, 109.6, 126.4, 126.9, 129.5, 134.7, 134.8, 144.6, 156.7, 156.8; EIMS, *m/z*: 322 (M − 2), 324 (M^+); Anal. Calcd. For $C_{14}H_{11}Cl_2N_3O_2$ (324.16): C, 51.87; H, 3.42; N, 12.96. Found: C, 52.64; H, 3.65; N, 13.12.

*4.1.1.3. 6-(2-Chloroethyl)-2-(4-chlorophenyl)-7-methyl-5H- [1,3,4] thiadiazolo [3,2-a]pyrimidin-5-one **(2c)**.* Buff crysyals, yield 60%; m.p. 198–200 °C; IR (KBr, cm^{-1}): 3065 (CH aromatic), 2924, 2854 (CH aliphatic), 1720 (C═O), 1500 (C═C); ^1H NMR (DMSO, 400 MHz): δ 2.51 (s, 3H, CH_3), 3.10 (t, 2H, CH_2–CH_2–Cl, J = 6.5 Hz), 3.89 (t, 2H, CH_2–CH_2–Cl, J = 6.5 Hz), 7.56 (d, 2H, aromatic H, J = 8.6 Hz), 7.79 (d, 2H, aromatic, J = 8.6 Hz); ^{13}C NMR (DMSO, 100 MH z) δ 15.4, 16.5, 62.0, 109.6, 126.4, 126.9, 129.5, 134.7, 134.7, 144.6, 156.5, 156.8; EIMS, *m/z*: 340 (M^+); Anal. Calcd. For $C_{14}H_{11}Cl_2N_3OS$ (340.23): C, 49.42; H, 3.26; N, 12.35. Found: C, 49.68; H,3.43; N, 12.49.

4.1.2. General procedure for the synthesis of compound (3a-l)

A mixture of compounds **2a**, **2b** or **2c** (0.002 mol) and appropriate secondary amine (0.002 mol) was dissolved in dry DMF and refluxed for 12h in presence of K_2CO_3 (0.004mol, 0.55 gm). Then the reaction mixture was poured over crushed ice, and the obtained solid was filtered, washed with water and crystallized with ethanol.

*4.1.2.1. 7-Methyl-6-(2-morpholinoethyl)-2-phenyl-5H- [1,3,4]oxadiazolo [3,2-a]pyrimidin-5-one **(3a)**.* Light brown crystals, yield 40%; m.p. 137–139 °C; IR (KBr, cm^{-1}): 3062 (CH aromatic), 2924, 2831 (CH aliphatic), 1705 (C═O); ^1H NMR ($CDCl_3$, 400 MHz): δ 2.19 (s, 3H, CH_3), 3.42 (t, 4H, morpholine −$N(CH_2)_2$, J = 4.7 Hz), 3.60 (t, 2H, CH_2, J = 4.7 Hz), 3.69 (t, 2H, CH_2, J = 4.7 Hz), 3.72 (t, 4H, morpholine $(CH_2)_2$–O, J = 4.7 Hz), 7.18 (d, 2H, aromactic H, J = 8.6 Hz), 7.26–7.47 (m, 3H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.7, 30.9, 45.8, 66.4, 67.2, 120.3, 127.9, 129.0, 129.2, 148.2, 148.9, 154.0, 160.8; EIMS, *m/z*: 340 (M^+). Anal. Calcd. For $C_{18}H_{20}N_4O_3$ (340.38): C, 63.52; H, 5.92; N, 16.46. Found: C, 63.70; H, 6.13; N, 16.72.

*4.1.2.2. 7-Methyl-2-phenyl-6-(2-(piperidin-1-yl)ethyl)-5H- [1,3,4] oxadiazolo [3,2-a]pyrimidin-5-one **(3b)**.* Dark brown crystals, yield 33%; m.p. 170–172 °C; IR (KBr, cm^{-1}): 3059 (CH aromatic), 2924, 2854 (CH aliphatic), 1705 (C═O); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.64 (s, 3H, CH_3), 1.66–1.71 (m, 10H, piperidineH), 3.34 (t, 2H, CH_2, J = 4.7 Hz), 3.55 (t, 2H, CH_2, J = 4.7 Hz), 7.41–7.49 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.7, 29.3, 29.7, 30.0, 55.2, 56.1, 121.0, 123.5, 124.3, 128.7, 131.3, 131.7, 144.8, 158.0, 160.0; EIMS, *m/z*: 338 (M^+), 339 (M^++1). Anal. Calcd. For $C_{19}H_{22}N_4O_2$ (338.40): C, 67.44; H, 6.55; N, 16.56. Found: C, 67.28; H, 6.71; N, 16.83.

*4.1.2.3. 7-Methyl-2-phenyl-6-(2-(4-phenylpiperazin-1-yl)ethyl)-5H-[1,3,4]oxadiazolo [3,2-a]pyrimidin-5-one **(3c)**.* Dark brown crystals, yield 42%; m.p. 167–169 °C; IR (KBr, cm^{-1}): 3059 (CH aromatic), 2924, 2823 (CH aliphatic), 1705 (C═O); ^1H NMR (DMSO, 400 MHz): 1.45 (s, 3H, CH_3), 3.17–3.23 (m, 8H, piperazine H), 3.56 (t, 2H, CH_2, J = 5.0 Hz), 3.74 (t, 2H, CH_2, J = 5.0 Hz), 6.96–7.00 (m, 5H, aromatic H), 7.31–7.85 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.5, 40.0, 45.6, 49.4, 50.5, 116.0, 116.5, 116.7, 117.1, 120.9, 128.5, 129.1, 129.3, 147.0, 150.9, 153.0, 159.0, 160.8; For EIMS, *m/z*: 415 (M^+); Anal. Calcd. For $C_{24}H_{25}N_5O_2$ (415.49): C, 69.38; H, 6.06; N, 16.86. Found: C, 69.49; H, 6.23; N, 17.04.

*4.1.2.4. 6-(2-(4-(4-Methoxyphenyl)piperazin-1-yl)ethyl)-7-methyl-2-phenyl-5H- [1,3,4]oxadiazolo [3,2-a]pyrimidin-5-one **(3d)**.* Buff crysyals, yield 38%; m.p. 195 °C; IR (KBr, cm^{-1}): 3059 (CH

aromatic), 2916, 2831 (CH aliphatic), 1701 (C═O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.13 (s, 3H, CH_3), 2.75 (s, 8H, piperazine 4CH_2), 3.15 (t, 2H, CH_2, J = 7.2 Hz), 3.84 (s, 3H, OCH_3), 4.34 (t, 2H, CH_2, J = 7.2 Hz), 7.51–7.53 (m, 5H, aromatic H), 7.85–7.87 (m, 4H, aromatic H); ^{13}C NMR (DMSO, 100 MHz): δ 16.09, 20.46, 22.69, 29.7, 55.7, 64.9, 117.1, 119.7, 123.6, 125.6, 126.7, 129.2, 131.2, 140.5, 142.5, 157.3, 160.3, 162.3; EIMS, *m/z*: 445 (M^+). Anal. Calcd. For $C_{25}H_{27}N_5O_3$ (445.51): C, 67.40; H, 6.11; N, 15.72. Found: C, 67.65; H, 6.24; N, 15.83.

4.1.2.5. 2-(4-Chlorophenyl)-7-methyl-6-(2-morpholinoethyl)-5H-[1,3,4]oxadiazolo [3,2-a]pyrimidin-5-one ***(3e)****.* Brown crystals, yield 70%; m.p. 155 °C; IR (KBr, cm^{-1}): 3066 (CH aromatic), 2924, 2854 (CH aliphatic), 1662 (C═O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.90 (s, 3H, CH_3), 3.42 (t, 2H, CH_2, J = 4.7 Hz), 3.59 (t, 2H, CH_2, J = 4.7 Hz), 3.68 (t, 4H, morpholine −N(CH_2)$_2$, J = 4.8 Hz), 3.72 (t, 4H, morpholine (CH_2)$_2$−O, J = 4.7 Hz), 7.36 (d, 2H, aromatic H, J = 8.8 Hz), 8.04 (d, 2H, aromatic H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.6, 27.0, 45.8, 47.2, 66.4, 121.0, 127.0, 128.0, 128.9, 130.5, 131.0, 160.9, 162.5; EIMS, *m/z*: 372 (M − 2), 374 (M^+); Anal. Calcd. For $C_{18}H_{19}ClN_4O_3$ (374.82): C, 57.68; H, 5.11; N, 14.95. Found: C, 57.92; H, 5.34; N, 15.19.

4.1.2.6. 2-(4-Chlorophenyl)-7-methyl-6-(2-(piperidin-1-yl)ethyl)-5H- [1,3,4]oxadiazolo [3,2-a]pyrimidin-5-one ***(3f)****.* Buff crysyals, yield 75%; m.p. 149 °C; IR (KBr, cm^{-1}): 3093, 3066 (CH aromatic), 2924, 2854 (CH aliphatic), 1639 (C═O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.43 (t, 4H, piperidine N(CH_2)$_2$, J = 7 Hz), 1.56–1.70 (m, 6H, piperidine H), 2.90 (s, 3H, CH_3), 3.32 (t, 2H, CH_2, J = 5.4 Hz), 3.50 (t, 2H, CH_2, J = 5.5 Hz), 7.30–7.52 (m, 4H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.6, 24.5, 26.0, 29.3, 52.5, 53.0, 126.9, 127.4, 128.3, 128.8, 129.0, 129.2, 129.4, 130.7, 161.0; EIMS, *m/z*: 372 (M^+); Anal. Calcd. For $C_{19}H_{21}ClN_4O_2$ (372.85): C, 61.21; H, 5.68; N, 15.03. Found: C, 60.97; H, 5.92; N, 15.21.

4.1.2.7. 2-(4-Chlorophenyl)-7-methyl-6-(2-(4-phenylpiperazin-1-yl) ethyl)-5H- [1,3,4]oxadiazolo [3,2-a]pyrimidin-5-one ***(3g)****.* Brown crystals, yield 60%; m.p. 178 °C; IR (KBr, cm^{-1}): 3093, 3059 (CH aromatic), 2924, 2823 (CH aliphatic), 1647 (C═O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.90 (s, 3H, CH_3), 3.17–3.23 (m, 8H, piperazine H), 3.49 (t, 2H, CH_2, J = 6.5 Hz), 3.73 (t, 2H, CH_2, J = 6.5 Hz), 6.95–7.43 (m, 9H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.8, 45.6, 49.3, 49.4, 50.8, 56.0, 116.5, 117.1, 120.9, 121.0, 124.0, 126.2, 126.8, 129.2, 129.3, 129.8, 133.0, 144.8, 160.8; EIMS, *m/z*: 449 (M^+); Anal. Calcd. For $C_{24}H_{24}ClN_5O_2$ (449.93): C, 64.07; H, 5.38; N, 15.57. Found: C, 64.31; H, 5.60; N, 15.68.

4.1.2.8. 2-(4-Chlorophenyl)-6-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl)-7-methyl-5H- [1,3,4]oxadiazolo [3,2-a]pyrimidin-5-one ***(3h)****.* Brown crystals, yield 63%; m.p. 158 °C; IR (KBr, cm^{-1}): 3066 (CH aromatic), 2920, 2850 (CH aliphatic), 1658 (C═O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.90 (s, 3H, CH_3), 3.04–3.08 (m, 8H, piperazine H), 3.49 (t, 2H, CH_2, J = 6.5 Hz), 3.72 (t, 2H, CH_2, J = 6.3 Hz), 3.79 (s, 3H, OCH_3), 6.86–7.49 (m, 8H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.7, 36.5, 45.8, 50.8, 55.5, 114.5, 118.0, 119.3, 127.0, 128.0, 128.1, 128.6, 130.0, 130.1, 137.5, 145.2, 154.6, 160.8; EIMS, *m/z*: 479 (M − 1), 480 (M^+); Anal. Calcd. For $C_{25}H_{26}ClN_5O_3$ (479.96): C, 62.56; H, 5.46; N, 14.59. Found: C, 62.78; H, 5.63; N, 14.73.

4.1.2.9. 2-(4-Chlorophenyl)-7-methyl-6-(2-morpholinoethyl)-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidin-5-one ***(3i)****.* Brown crystals, yield 65%; m.p. 171 °C; IR (KBr, cm^{-1}): 3089 (CH aromatic), 2924, 2854 (CH aliphatic), 1662 (C═O); ^{1}H NMR (DMSO, 400 MHz): δ 2.89 (s, 3H, CH_3), 3.50 (t, 2H, CH_2, J = 4.8 Hz), 3.58 (broad s, 8H, morpholine H), 3.73 (t, 2H, CH_2, J = 5.0 Hz), 7.50–7.65 (m, 4H, aromatic H); ^{13}C NMR (DMSO, 100 MHz): δ 23.4, 27.0, 50.0, 60.0, 66.1, 112.8, 116.0, 118.1, 123.0, 126.2, 128.1, 129.0, 130.0, 162.3, 169.0; EIMS, *m/z*: 390 (M^+), 392 (M^++2); Anal. Calcd. For $C_{18}H_{19}ClN_4O_2S$ (390.89): C, 55.31; H, 4.90; N, 14.33. Found: C, 55.49; H, 4.82; N, 14.25.

4.1.2.10. 2-(4-Chlorophenyl)-7-methyl-6-(2-(piperidin-1-yl)ethyl)-5H- [1,3,4]thiadiazolo [3,2-a]pyrimidin-5-one ***(3j)****.* Brown crystals, yield 53%; m.p. 120 °C; IR (KBr, cm^{-1}): 3086 (CH aromatic), 2935, 2854 (CH aliphatic), 1670 (C═O); ^{1}H NMR (DMSO, 400 MHz): δ 1.45–1.58 (m, 10H, piperidine H), 2.36 (t, 2H, CH_2 J = 5.4 Hz), 2.73 (t, 2H, CH_2, J = 5.4 Hz), 2.89 (s, 3H, CH_3), 7.48–7.97 (m, 4H, aromatic H); ^{13}C NMR (DMSO, 100 MHz): δ 21.5, 22.0, 23.1, 24.5, 53.5, 55.6, 127.9, 128.3, 129.5, 129.6, 130.0, 130.1, 134.5, 160.1, 169.3; EIMS, *m/z*: 388 (M^+); Anal. Calcd. For $C_{19}H_{21}ClN_4OS$ (388.91): C, 58.68; H, 5.44; N, 14.41. Found: C, 58.91; H, 5.67; N, 14.68.

4.1.2.11. 2-(4-Chlorophenyl)-7-methyl-6-(2-(4-phenylpiperazin-1-yl) ethyl)-5H- [1,3,4]thiadiazolo [3,2-a]pyrimidin-5-one ***(3k)****.* Dark brown crystals, yield 43%; m.p. 156 °C; IR (KBr, cm^{-1}): 3089, 3059 (CH aromatic), 2885, 2819 (CH aliphatic), 1658 (C═O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.98 (s, 3H, CH_3), 3.23–3.37 (m, 8H, piperazineH), 3.56 (t, 2H, CH_2, J = 6.5 Hz), 3.80 (t, 2H, CH_2, J = 6.3 Hz), 6.95–7.71 (m, 9H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.5, 36.5, 45.9, 49.4, 50.9, 116.0, 116.7, 117.1, 120.0, 120.9, 127.9, 128.0, 128.7, 128.8, 129.3, 145.9, 150.0, 161.0; EIMS, *m/z*: 465 (M − 1), 467 (M^++1); Anal. Calcd. For $C_{24}H_{24}ClN_5OS$ (466.00): C, 61.86; H, 5.19; N, 15.03. Found: C, 61.74; H,5.28; N, 15.29.

4.1.2.12. 2-(4-Chlorophenyl)-6-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl)-7-methyl-5H- [1,3,4]thiadiazolo [3,2-a]pyrimidin-5-one ***(3l)****.* Brown crystals, yield 40%; m.p. 153 °C; IR (KBr, cm^{-1}): 3063 (CH aromatic), 2931, 2831 (CH aliphatic), 1700 (C═O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.970 (s, 3H, CH), 2.97–3.11 (m, 10 H, piperazine H, CH_2), 3.56 (t, 2H, CH_2, J = 6.5 Hz), 3.79 (s, 3H, OCH_3), 6.88–7.77 (m, 8H, aromatic H); EIMS, *m/z*: 497 (M^++1), 499 (M^++3); Anal. Calcd. For $C_{25}H_{26}ClN_5O_2S$ (496.02): C, 60.53; H, 5.28; N, 14.12. Found: C, 60.79; H, 5.41; N, 14.34.

4.2. Biological evaluation

4.2.1. In vivo screening

All the pharmacological *in vivo* study procedures obeyed the standards and animals' treatment protocol was approved by the Research Ethics Committee, Faculty of Pharmacy, Cairo University, Egypt (number PC: 2949). All the experiments were performed in accordance with the relevant guidelines and regulations.

4.2.1.1. Analgesic activity. All new final compounds were screened for their analgesic activity using *p*-benzoquinone-induced writhing method in mice adopted by Okun et al. [29] using indomethacin as a reference standard. Adult male albino mice (obtained from National Research Centre) weighing 20–25 g were used in this study. The target compounds as well as indomethacin reference standard were prepared as suspension in 2% Tween 80.

The animals were kept in the animal house under standard laboratory conditions of light and temperature. All animals were accessed to standard laboratory diet. In all tests, adequate considerations were adopted to reduce pain or discomfort of animals. A sensitivity test was carried out one day before drug administration, where the animals were injected intraperitoneally with 0.20–0.25 ml of 0.02% freshly prepared solution of *p*-benzoquinone in distilled water. Animals showing writhing to *p*-benzoquinone within 30 min were chosen for studying the analgesic activity [39].

On the next day, mice were divided into 16 groups, 6 animals each. The control group received only 2% Tween 80, while the rest of the groups received the reference standard and the tested

compounds at a dose of 28 μm/kg body weight. An hour later, 0.02% solution of *p*-benzoquinone was administered intraperitoneally, the animals were observed for 30 min after injection of the irritant, and the animals showing writhing were counted in each group. Writhing is known as stretch, torsion to one side, drawing up of hind leg, retraction of the abdomen, so that the belly of mouse touches the floor. The mice showing any of the previous signs were counted as positive responses and this method depends on the ability of the tested compounds to protect the animals from writhing signs made by *p*-benzoquinone.

The analgesic activity was evaluated as the percentage protection of tested animals against irritant *p*-benzoquinone induced writhing response compared with the control group according the following equation:

$$\% \text{ Protection } = \frac{\text{Number of protected animals x 100}}{\text{Total number of animals}}$$

The % potency of the tested compounds was expressed as % protection for the tested compounds relative to % protection for indomethacin according to the following equation [37,40]:

$$\% \text{ Potency} = \frac{\% \text{ protection for the tested compound treated group}}{\% \text{ protection for indomethacin treated group}}$$

The paw edema was induced by suplantar injection of 0.1 ml 1% carrageenan solution in saline (0.9%). Paw edema volume was measured after 2 and 3 h using the plethysmomoeter and compared with the initial hind paw volume of each rat. The difference of average values between treated and control group is calculated for each time interval and evaluated statistically. Quantitative variables from normal distribution were expressed as mean ± standard error (SEM). The anti-inflammatory activity was expressed as percentage inhibition of edema volume in treated animals in comparison with the control group according to the following equation:

$$\% \text{ Inhibition } = \frac{\text{Vc} - \text{Vt}}{\text{Vc}} \text{x } 100$$

where **Vc** is the mean of edema volume of rat paw after administration of carrageenan in the control group, **Vt** is the mean of edema volume of rat paw after administration of the tested compounds or the reference drug.

The %potency of the tested compounds was expressed as % inhibition of edema thickness for the tested compounds relative to % inhibition of edema for indomethacin "reference standard" at 2h effect (the time at which indomethacin reveals its maximum bioproperties) according to the following equation [37,40]:

$$\% \text{ Potency } = \frac{\% \text{ inhibtion of edema for the tested compound treated group}}{\% \text{ inhibtion of edema for indomethacin treated group}}$$

4.2.1.2. Toxicological study. Toxicological study of the most active compounds **3b**, **3d**, **3g**, and **3h** was determined using Finney's method [30]. Adult male albino mice (obtained from National Research Centre) weighing 20–25g were used in this study. Mice were divided into 6 groups each of six animals. The control group received only 2% Tween 80, while the rest of the groups received the reference standard and the tested compounds. Minimal dose that killed all animals and the maximal dose that failed to kill any animal were determined *via* several increasing intraperitoneal doses up to 5 folds of the used analgesic dosage. Animal were kept under observation for 24hr during which any mortality in each group were recorded.

4.2.1.3. Anti-inflammatory activity. Compounds **3b**, **3d**, **3g**, and **3h** that showed the best analgesic activity were screened for their anti-inflammatory activity using Carrageenan-induced rat paw edema method reported by Winter et al. [31] using indomethacin as reference standard. This technique is used to test the ability of the tested compounds to reduce the edema produced in the paw of the rat after injection with carrageenan.

Male Wister albino rats (obtained from National Research Centre) weighing 100–120 gm were used. The rats were kept in the animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into 6 groups of five rats each. The initial hind paw volume of rats was determined volumetrically by means of plethysmometer 7150 (UGO Basile, Italy). The suspended tested compounds and the reference standard indomethacin in 2% Tween 80 were administered intraperitoneally at a dose of 28 μm/kg body weight, while the control group received only 2% Tween 80, 1h before induction of inflammation.

4.2.1.4. Ulcerogenic liability. The ulcerogenic effect of compounds **3b**, **3d**, **3g**, and **3h** that were exhibited a promising analgesic and anti-inflammatory activities and reference drug indomethacin was evaluated by the reported method of Meshali et al. [32] Adult male albino rats (obtained from National Research Centre) weighing 100–120 g were used in this study. Animals were fasted 18 h before the drug administration, then divided into 5 groups each of 5 animals and received the drug orally. The first group received 2% tween 80 and kept as control, the second group received indomethacin in a dose of 28 μm/kg body weight and the rest of the groups were received 4 tested compounds in the same dose.

Food was allowed 2h after administration of the drugs and rats received the same dose orally for three successive days. Two hours after the last dose, rats were sacrificed, the stomach of each rat were removed, opened along greater curvature and cleaned by washing with cold saline. The stomach was stretched on a cork-board using pins and examined with a magnifying lens (10×) for the presence ulcers and erosions. Ulcer index was calculated according to the method of Robert et al. [33]. The degree of ulcerogenic effect was expressed in term of; percentage incidence of ulcer in each group of animals divided by 10, the average number of ulcers per stomach and the average severity of ulcers by visual observation. The ulcer index was expressed as summation value of the above three values.

4.2.2. In vitro COX inhibition assay

Selected most active compounds **3b**, **3d**, **3g**, and **3h**, in addition to the reference drugs celecoxib and indomethacin were tested for their ability to inhibit COX-1 and COX-2 using BioVision's COX-1 Inhibitor Screening Kit (Fluorometric) Cat. #K548 and COX-2 Inhibitor Screening Kit (Fluorometric) Cat. #K547 that offer a rapid, simple, sensitive, and reliable test suitable for high-throughput

screening of COX-1 and COX-2 inhibitors by an assay based on the fluorometric detection of Prostaglandin G2, the intermediate product generated by the COX enzyme; assay was performed following the kit protocol. Results of experiments were recorded as a mean of triplicates ± SEM. IC_{50} values were calculated by GraphPad Prism5 software; data fit was obtained using nonlinear regression dose−response-inhibition equation (variable slope).

4.3. Physicochemical parameters

In order to predict physicochemical parameters of all the target compounds, computational study has been performed. Molecular weight, logP, number of hydrogen bond acceptor and donor atoms of Lipinski's "rule of five" [25]. Additionally, number of rotatable bonds and topological polar surface area (TPSA) were calculated using the molinspiration online property calculation toolkit [41]. The computed molecular properties are recorded in Table S1. The degree of absorption is expressed as the percentage of absorption (%ABS), that was calculated as %ABS = 109 - (0.345 x TPSA).

4.4. In silico studies

4.4.1. 2D-QSAR study

2D-QSAR studies for the tested conjugated (**3a-3l**) were undertaken to utilize the comprehensive descriptors for structural and statistical analysis (CODESSA-Pro) software [34] (see supplementary material).

4.4.2. 3D pharmacophore study

The synthesized agents with potential analgesic properties (**3a-3l**) were undertaken by Discovery Studio 2.5 software for identifying the 3D-pharmacophic hypothesis describing the bio-observations (standard technique, structure optimization by CHARMm, partial charge by Momany-Rone).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The Authors would like to thank Prof. Dr. Adel S. Girgis Department of Pesticide Chemistry, National Research Centre for carrying out the computational studies and thank members of Pharmacology and Toxicology Department, Faculty of Pharmacy, Cairo University for helping during carrying out the *in vivo* study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113682.

References

[1] H.H. Hassanein, H.H. Georgey, M.A. Fouad, A.M.E. Kerdawy, M.F. Said, Synthesis and molecular docking of new imidazoquinazolinones as analgesic agents and selective COX-2 inhibitors, Future Med. Chem. 9 (2017) 553−578, https://doi.org/10.4155/fmc-2016-0240.
[2] E.M. Gedawy, A.E. Kassab, A.M.E. Kerdawy, Design, synthesis and biological evaluation of novel pyrazole sulfonamide derivatives as dual COX-2/5-LOX inhibitors, Eur. J. Med. Chem. 189 (2020) 112066, https://doi.org/10.1016/j.ejmech.2020.112066.
[3] F.A. Ragab, N.M.A. Gawad, H.H. Georgey, M.F. Said, Synthesis of novel 1,3,4-trisubstituted pyrazoles as anti-inflammatory and analgesic agents, Eur. J. Med. Chem. 63 (2013) 645−654, https://doi.org/10.1016/j.ejmech.2013.03.005.
[4] R. Borne, M. Levi, N. Wilson, Nonsteroidal anti-inflammatory drugs, in: T.L. Lemke, D.A. Williams (Eds.), Foye's Principles of Medicinal Chemistry, Wolters Kluwer, Lippincott Williams & Wilkins, USA, 2008.
[5] F.A.-F. Ragab, N.M. Abdel-Gawad, H.H. Georgey, M.F. Said, Pyrazolone derivatives: synthesis, anti-inflammatory, analgesic, quantitative structure−activity relationship and in vitro studies, Chem. Pharm. Bull. 61 (2013) 834−845, https://doi.org/10.1248/cpb.c13-00314.
[6] C.J. Friel, M.C. Lu, J. John, M. Beale, J.H. Block, Analgesics, in: Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, Williams & Wilkins, a Wolters Kluwer business Lippincott, 2011.
[7] S. Fiorucci, R. Meli, M. Bucci, G. Cirino, Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy? Biochem. Pharmacol. 62 (2001) 1433−1438.
[8] J.R.D. Vane, Frs, R.M.P. Botting, Mechanism of action of nonsteroidal antiinflammatory drugs, Am. J. Med. 104 (1998) 2S−8S, https://doi.org/10.1016/s0002-9343(97)00203-9.
[9] H.U. Zeilhofer, Prostanoids in nociception and pain, Biochem. Pharmacol. 73 (2007) 165−174, https://doi.org/10.1016/j.bcp.2006.07.037.
[10] S.M. Stillman, Mj, Choosing nonselective NSAIDs and selective COX-2 inhibitors in the elderly. A clinical use pathway, Geriatrics 62 (2007) 26−34.
[11] D. Bishop-Bailey, J.A. Mitchell, T.D. Warner, COX-2 in cardiovascular disease, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 956−958, https://doi.org/10.1161/01.ATV.0000219672.68024.bc.
[12] R.P. Mason, M.F. Walter, C.A. Day, R.F. Jacob, A biological rationale for the cardiotoxic effects of rofecoxib: comparative analysis with other COX-2 selective agents and NSAIDs, Subcell. Biochem. 42 (2007) 175−190.
[13] J.V. Ryn, G. Trummlitz, M. Pairet, COX-2 selectivity and inflammatory processes, Curr. Med. Chem. 7 (2000) 1145−1161, https://doi.org/10.2174/0929867003374255.
[14] G. Dannhardt, S. Laufer, Structural approaches to explain the selectivity of COX-2 inhibitors: is there a common pharmacophore ? Curr. Med. Chem. 7 (2000) 1101−1112, https://doi.org/10.2174/0929867003374237.
[15] J.K. Gupta, P.K. Sharma, R. Dudhe, A. Chaudhary, P.K. Verma, Synthesis and analgesic activity of novel pyrimidine derivatives of coumarin moiety, Analele UniversitáÑii din Bucuresti 19 (2010) 9−21.
[16] H.N. Hafez, H.-A.S. Abbas, A.-R.B.A. EL-Gazzar, Synthesis and evaluation of analgesic, anti-inflammatory and ulcerogenic activities of some triazolo- and 2-pyrazolyl-pyrido[2,3-d]-pyrimidines, Acta Pharm. 58 (2008) 359−378, https://doi.org/10.2478/v10007-008-0024-1.
[17] A.A. Bekhit, H.T.Y. Fahmy, S.A.F. Rostom, A.M. Baraka, Design and synthesis of some substituted 1*H*-pyrazolyl-thiazolo[4,5-*d*]pyrimidines as anti-inflammatory-antimicrobial Agents, Eur. J. Med. Chem. 38 (2003) 27−36, https://doi.org/10.1016/s0223-5234(02)00009-0.
[18] E.K.A. Abdelall, P.F. Lamie, A.K.M. Ahmed, E.-S. EL-Nahass, COX-1/COX-2 inhibition assays and histopathological study of the new designed anti-inflammatory agent with a pyrazolopyrimidine core, Bioorg. Chem. 86 (2019) 235−253, https://doi.org/10.1016/j.bioorg.2019.01.031.
[19] M. Akhter, N. Akhter, M.M. Alam, M.S. Zaman, R. Saha, A. Kumar, Synthesis and biological evaluation of 2,5-disubstituted1,3,4-oxadiazole derivatives with both COX and LOX inhibitory activity, J. Enzym. Inhib. Med. Chem. 26 (2011) 767−776, https://doi.org/10.3109/14756366.2010.550890.
[20] G. Chawla, U. Kumar, S. Bawa, J. Kumar, Syntheses and evaluation of anti-inflammatory, analgesic and ulcerogenic activities of 1,3,4-oxadiazole and 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole derivatives, J. Enzym. Inhib. Med. Chem. 27 (2012) 658−665, https://doi.org/10.3109/14756366.2011.606543.
[21] G.C. Ramaprasad, B. Kalluraya, B.S. Kumar, S. Mallya, Synthesis of new oxadiazole derivatives as anti-inflammatory, analgesic, and antimicrobial agents, Med. Chem. Res. 22 (2013) 5381−5389, https://doi.org/10.1007/s00044-012-0298-1.
[22] F.A. Ragab, H.I. Heiba, M.G. El-Gazzar, S.M. Abou-Seri, W.A. El-Sabbagh, R.M. El-Hazekb, Synthesis of novel thiadiazole derivatives as selective COX-2 inhibitors, Med. Chem. Commun 7 (2016) 2309, https://doi.org/10.1039/c6md00367b.
[23] V.B. Jadhav, M.V. Kulkarni, V.P. Rasal, S.S. Biradar, M.D. Vinay, Synthesis and anti-inflammatory evaluation of methylene bridged benzofuranyl imidazo [2,1-b][1,3,4]thiadiazoles, Eur. J. Med. Chem. 43 (2008) 1721−1729, https://doi.org/10.1016/j.ejmech.2007.06.023.
[24] M.T. Alam, Abida, M. Asif, A review on analgesic and anti-inflammatory activities of various piperazinyl containing pyridazine derivatives, Prog. Chem. Biochem. Res. 3 (2020) 81−92, https://doi.org/10.33945/SAMI/PCBR.2020.2.1.
[25] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (2001) 3−26, https://doi.org/10.1016/s0169-409x(00)00129-0.
[26] H. Rajak, A. Agarawal, P. Parmar, B.S. Thakur, R. Veerasamy, P.C. Sharma, M.D. Kharya, 2,5-Disubstituted-1,3,4-oxadiazoles/thiadiazole as surface recognition moiety: design and synthesis of novel hydroxamic acid based histone deacetylase inhibitors, Bioorg. Med. Chem. Lett 21 (2011) 5735−5738, https://doi.org/10.1016/j.bmcl.2011.08.022.
[27] P. Mishra, H. Rajak, A. Mehta, Synthesis of Schiff bases of 2-amino-5-aryl-1,3,4-oxadiazoles and their evaluation for antimicrobial activities, J. Gen. Appl. Microbiol. 51 (2005) 133−141, https://doi.org/10.2323/jgam.51.133.
[28] V. Jatav, P. Mishra, S. Kashaw, J. Stables, Synthesis and CNS depressant activity of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-

4(3H)-ones, Eur. J. Med. Chem. 43 (2008) 135–141, https://doi.org/10.1016/j.ejmech.2007.02.004.
[29] R. Okun, S.C. Lidddon, L. Lasagna, The effect of aggregation, electric shock and adrenergic bloking drugs on inhibition of the "writhing syndrome, J. Pharmacol. Exp. Therapeut. 139 (1963) 107–109.
[30] D.J. Finney, Statistical Methods in Biological Assay, second ed., Griffin, London, 1964, p. 597.
[31] C.A. Winter, E.A. Risely, G.M. Nuss, Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547, https://doi.org/10.3181/00379727-111-27849.
[32] M. Meshali, E. El-Sabbagh, A. Foda, Effect of encapsulation of flufenamic acid with acrylic resins on its bioavailability and gastric ulcerogenic activity in rats, Acta Pharm. Technol. 29 (1983) 217–230.
[33] A. Robert, J.E. Nezamis, J.P. Phillips, Effect of prostaglandin E1 on gastric secretion and ulcer formation in the rat, Gastroenterology 55 (1968) 481–487, https://doi.org/10.1016/S0016-5085(19)34024-7.
[34] A.R. Katritzky, R. Petrukhin, I. Petrukhina, A. Lomaka, D.B. Tatham, M. Karelson, CODESSA-Pro software manual 52 (72) (2005) 77.
[35] M.N. Aziz, S.S. Panda, E.M. Shalaby, N.G. Fawzy, A.S. Girgis, Facile synthetic approach towards vasorelaxant active 4-hydroxyquinazoline-4-carboxamides, RSC Adv. 9 (2019) 28534–28540, https://doi.org/10.1039/c9ra04321g.
[36] A.S. Girgis, S.S. Panda, A.M. Srour, H. Farag, N.S.M. Ismail, M. Elgendy, A.K. Abdel-Aziz, A.R. Katritzky, Rational design, synthesis and molecular modeling studies of novel anti-oncological alkaloids against melanoma, Org. Biomol. Chem. 13 (2015) 6619–6633, https://doi.org/10.1039/C5OB00410A.
[37] S.S. Panda, A.S. Girgis, H.H. Honkanadavar, R.F. George, A.M. Srour, Synthesis of new ibuprofen hybrid conjugates as potential anti-inflammatory and analgesic agents, Future Med. Chem. 12 (2020) 1369–1386, https://doi.org/10.4155/fmc-2020-0109.
[38] N.G. Fawzy, S.S. Panda, W. Fayad, E.M. Shalaby, A.M. Srour, A.S. Girgis, Synthesis, human topoisomerase IIα inhibitory properties and molecular modeling studies of anti-proliferative curcumin mimics, RSC Adv. 9 (2019) 33761–33774, https://doi.org/10.1039/C9RA05661K.
[39] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, W.C. Stalling, Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, Nature 384 (1996) 644–648, https://doi.org/10.1038/384644a0.
[40] R.N. Naumov, S.S. Panda, A.S. Girgis, R.F. George, M. Farhat, A.R. Katritzky, Synthesis and QSAR study of novel anti-inflammatory active mesalazine-metronidazole conjugates, Bioorg. Med. Chem. Lett 25 (2015) 2314–2320, https://doi.org/10.1016/j.bmcl.2015.04.023.
[41] Molinspiration cheminformatics. http://www.molinspiration.com/services/properties.html accessed on 24 March 2014.